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(54) Title: NOVEL PROCOAGULANT PROTEINS

(57) Abstract

Novel procoagulant proteins which comprise the amino acid sequence: A-X-B wherein region A represents the polypeptide sequence Ala-20 through Arg-759 substantially as shown in Table 1; region B represents the polypeptide sequence Ser-1709 through Tyr-2351 substantially as shown in Table 1; and region X represents a polypeptide sequence comprising up to 949 amino acids substantially duplicative of sequences of amino acids within the sequence Ser-760 through Arg-1708 of Table 1, wherein the amino terminus of X is covalently bonded through a peptide bond designated '-' to the carboxy terminus of A, and the carboxy terminus of X is likewise bonded to the amino terminus of B. Methods of making such proteins and their use in pharmaceutical preparations is also disclosed.

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203

124

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195

235

NOVEL PROCOAGULANT PROTEINS

This invention relates to a novel series of proteins which exhibit procoagulant properties. These proteins have marked structural differences from human factor VIII:C, but have similar procoagulant activity.

Factor VIII:C is the blood plasma protein that is defective or absent in Hemophilia A disease. This disease is a hereditary bleeding disorder affecting approximately one in 20,000 males.
10The structure of factor VIII:C is described in U.S. Patent Applications Serial No. 546,650 filed October 28, 1983 and No. 644,036 filed August 24, 1984, which are incorporated herein by reference and in Nature, 312:306, 307, 326 and 342.

15One of the problems presently encountered with the use of human factor VIII:C for treatment of hemophilia arises from its antigenicity. A significant percentage of hemophiliacs have developed an immune reaction to the factor VIII:C used for their treatment. Non-hemophiliacs can also develop or acquire 20hemophilia when their immune systems become sensitized to factor VIII:C and produce circulating antibodies or "inhibitors" to factor VIII:C. In either case, the effect is the neutralization of whatever factor VIII:C is present in the patient, making treatment very difficult. Until now, the method of 25choice for treating hemophiliacs with this problem has been to administer, in cases of severe bleeding episodes, non-human factor VIII:C, such as treated porcine factor VIII:C. See Kernoff et al., Blood 63:31 (1984). However, the antibodies which neutralize the clotting ability of human factor VIII:C 30will react to a varying extent with factor VIII:C of other species, and the porcine protein is itself antigenic, thus both the short-term and long-term effectiveness of such treatment will vary.

164

209

Additionally, patients frequently display adverse reactions to infusion with the porcine factor VIII:C. The use of porcine factor VIII:C in spite of the risks has been justified because of the lack of reliably effective alternatives. Kernoff,
5 supra at 38. The present invention provides an alternative to the administration of porcine factor VIII:C.

This invention provides for proteins which have procoagulant activity similar to that of factor VIII:C and also have substantially lower molecular weight. These proteins are schematically depicted by formula (1) as follows:

(1) A-X-B

15 wherein A represents a polypeptide sequence substantially duplicative of the sequence Ala-20 through Arg-759; B represents a polypeptide sequence substantially duplicative of the sequence Ser-1709 through the C-terminal Tyr-2351; and X represents a polypeptide sequence of up to 949 amino acids substantially 20 duplicative of sequences of amino acids within the sequence Ser-760 through Arg-1708. The amino terminus of region X is covalently bonded through a peptide bond (designated "-" in formula 1) to the carboxy terminus of A. The carboxy terminus of region X is likewise bonded to the amino terminus of B.
25 Numbering of amino acids throughout this disclosure is with reference to the numbering of amino acids in Table 1 in which the first amino acid, Met, of the leader sequence is assigned Number 1. Protein domain X may comprise a continuous but shorter sequence selected from the region Ser-760 through
30 Arg-1708. Alternatively X may comprise two or more amino acid sequences selected from that region which are covalently bonded by a peptide bond (maintaining an ascending numerical order of amino acids).

35 By way of example, one compound of this invention contains a region X comprising the amino acid sequence of Ser-760 to Pro-

167
-240

TABLE 1

5' GAATTCCCCACTGGCTAACCTCCCTAAATGCTCTGGAAAGAAATTCCGACTTTCACTAAATCACAAATT																									
TTACTTTTCCCCTCCCTGGACCTAAACATATTTAGACAAGAATTAAACCTTTGCTTCAGTTAACATTGTAGCAATAACTC																									
MET	Cln	Ile	Glu	Leu	Ser	Thr	Cys	Phe	Phe	Leu	Cys	Leu	Leu	Arg	Phe	Cys	Phe							18	
ATG	CAA	ATA	GAG	CTC	TCC	ACC	TGC	TTC	TTT	CTG	TGC	CTT	ITG	CCA	ITC	TCC	TCC	TTT							
Ser	Ala	Thr	Arg	Arg	Tyr	Tyr	Leu	Gly	Ala	Val	Glu	Leu	Ser	Trp	Asp	Tyr	MET							36	
ACT	CCC	ACC	ACA	ACA	TAC	TAC	CTG	CGT	CCA	CTG	CAA	CTG	TCA	TGG	GAC	TAT	ATC								
Gln	Ser	Asp	Leu	Gly	Glu	Leu	Pro	Val	Asp	Ala	Arg	Phe	Pro	Pro	Arg	Val	Pro							54	
CAA	AGT	GAT	CTC	CGT	GAG	CTG	CCT	GTG	CAC	CCA	AGA	TTT	CCT	CCT	AGA	CTG	CCA								
Lys	Ser	Phe	Pro	Phe	Aen	Thr	Ser	Val	Val	Tyr	Lys	Lys	Thr	Leu	Phe	Val	Glu							72	
AAA	TCT	TTT	CCA	ITC	AAC	ACC	TCA	CTC	CTG	TAC	AAA	AAG	ACT	CTG	ITT	CTA	CAA								
Phe	Thr	Val	His	Leu	Phe	Asn	Ile	Ala	Lys	Pro	Arg	Pro	Pro	Trp	MET	Gly	Leu							90	
ITC	ACG	CTT	CAC	CTT	ITC	AAC	ATC	CCT	AAG	CCA	AGG	CCA	CCC	TGG	ATC	CCT									
Leu	Gly	Pro	Thr	Ile	Gln	Ala	Glu	Val	Tyr	Asp	Thr	Val	Val	Ile	Thr	Leu	Lys							108	
CTA	CGT	CCT	ACC	ATC	CAG	CCT	GAG	CTT	TAT	CAT	ACA	GTC	GTC	ATT	ACA	CTT									
Asn	MET	Ala	Ser	His	Pro	Val	Ser	Leu	His	Ala	Val	Gly	Val	Ser	Tyr	Trp	Lys							126	
AAC	ATG	GCT	TCC	CAT	CCT	GTC	ACT	CTT	CAT	CCT	GTT	GTA	TCC	TAC	TGG		AAA								
Ala	Ser	Glu	Gly	Ala	Glu	Tyr	Asp	Asp	Gin	Thr	Ser	Gln	Arg	Glu	Lys	Glu	Asp							144	
GCT	TCT	GAG	GGA	GCT	GAA	TAT	CAT	GAT	CAC	ACC	ACT	CAA	AGG	GAG	AAA	CAA	CAT								
Asp	Lys	Val	Phe	Pro	Gly	Gly	Ser	His	Thr	Tyr	Val	Trp	Gln	Val	Leu	Lys	Glu							162	
CAT	AAA	GTC	TTC	CCT	GGT	CGA	ACC	CAT	ACA	TAT	CTC	TGG	CAG	CTC	CTG	AAA									
Asn	Gly	Pro	MET	Ala	Ser	Asp	Pro	Leu	Cys	Leu	Thr	Tyr	Ser	Tyr	Leu	Ser	His							180	
AAT	CGT	CCA	ATG	CCC	TCT	CAC	CCA	CTG	TCC	CTT	ACC	TAC	TCA	TAT	TCT		CAT								
Val	Asp	Leu	Val	Lys	Asp	Leu	Asn	Ser	Gly	Leu	Ile	Gly	Ala	Leu	Leu	Val	Cys							198	
GTC	GAC	CTG	CTA	AAA	GAC	TTG	AAT	TCA	GGC	CTC	ATT	GGA	CCC	CTA	CTA		TGT								
Arg	Glu	Gly	Ser	Leu	Ala	Lys	Glu	Lys	Thr	Gln	Thr	Leu	His	Lys	Phe	Ile	Leu							216	
ACA	CAA	GGG	ACT	CTG	CCC	AAC	AAU	CAA	AAG	ACA	CAC	ACC	TTG	CAC	AAA	TTT	ATA	CTA							
Leu	Phe	Ala	Val	Phe	Asp	Glu	Gly	Lys	Ser	Trp	His	Ser	Glu	Thr	Lys	Asn	Ser							234	
CTT	TTT	GCT	GTA	TTT	CAT	GAA	GGG	AAA	AGT	TGG	CAC	TCA	GAA	ACA	AAG	AAC	TCC								
Leu	MET	Cln	Asp	Arg	Asp	Ala	Ala	Ser	Ala	Arg	Ala	Trp	Pro	Lys	MET	His	Thr							252	
TTC	ATG	CAG	GAT	AGG	GAT	GCT	CCA	TCT	CCT	CCG	UCC	TGG	CCT	AAA	ATG	CAC									
Val	Asn	Gly	Tyr	Val	Asn	Arg	Ser	Leu	Pro	Gly	Leu	Ile	Gly	Cys	His	Arg	Lys							270	
GTC	AAT	CGT	TAT	GTA	AAC	AGG	TCT	CTG	CCA	GGT	CTG	ATT	CGA	TCC	CAC	AGG	AAA								
Ser	Val	Tyr	Trp	His	Val	Ile	Gly	MET	Gly	Thr	Thr	Pro	Glu	Val	His	Ser	Ile							288	
TCA	CTC	TAT	TCC	CAT	GTC	ATT	GCA	ATG	GGC	ACC	ACT	CCT	GAA	GTC	CAC	TCA	ATA								
Phe	Lue	Glu	Gly	His	Thr	Phe	Leu	Val	Arg	Asn	His	Arg	Cln	Ala	Ser	Leu	Glu							306	
ITC	CTC	CAA	GCT	CAC	ACA	TTT	CTT	CTG	Arg	ACC	AAC	CAC	CCC	TCC	CGG	TTG									

168

211

TABLE 1, continued

Ile	Ser	Pro	Ile	Thr	Phe	Ileu	The	Ala	Gln	The	Ileu	Ileu	MET	Asp	Ileu	Gly	Gln	324
ATC	TCG	CCA	ATA	ACT	TTC	CTT	ACT	CCT	CAA	ACA	CTC	TTG	ATC	CAC	CTT	CAA	CAG	
Phe	Leu	Leu	Phe	Cys	His	Ile	Ser	Ser	Gln	His	Gln	Asp	Gly	MET	Glu	Ala	Tyr	342
TTT	CTA	CTG	TTT	TCT	CAT	ATC	TCT	TCC	CAC	CAA	CAT	GAT	CCC	ATG	CAA	GCT	TAT	
Val	Lys	Val	Asp	Ser	Cys	Pro	Glu	Glu	Pro	Gln	Ileu	Arg	MET	Lys	Asn	Asn	Glu	360
GTC	AAA	GTA	GAC	ACC	TGT	CCA	GAG	GAA	CCC	CAA	CTA	CCA	ATG	AAA	AAT	AAT	CAA	
Glu	Ala.	Glu	Asp	Tyr	Asp	Asp	Asp	Glu	Leu	Thr	Asp	Ser	Clu	MET	Asp	Val	Arg	378
CAA	CCC	CAA	GAC	TAT	GAT	GAT	GAT	GAG	CTT	ACT	GAT	ICT	GAA	ATG	GAT	GTC	AGG	
Phe	Asp	Asp	Asp	Asn	Ser	Pro	Ser	Phe	Ile	Gln	Ile	Arg	Ser	Val	Ala	Lys	Lys	396
TTT	GAT	GAT	GAC	AAC	TCT	CCT	TCC	TTT	ATC	CAA	ATT	CCC	TCA	GTC	GCU	AAC	AAC	
His	Pro	Lys	Thr	Trp	Val	His	Tyr	Ile	Ala	Glu	Glu	Glu	GAC	Irp	TGG	Asp	Tyr	414
CAT	CCT	AAA	ACT	IGG	GTA	CAT	TAC	ATT	GCT	GAA	GAG	GAG	ATC	TCC	GAC	TAT	TAT	
Ala	Pro	Leu	Val	Leu	Ala	Pro	Asp	Asp	Arg	Ser	Tyr	Lys	Ser	Gln	Tyr	Leu	Asn	432
GCT	CCC	TTA	GTC	CTC	GCC	CCC	GAT	GAT	ACA	AGT	TAT	AAA	AGT	CAA	TAT	TTG	AAC	
Asn	Gly	Pro	Gln	Arg	Ile	Gly	Arg	Lys	Tyr	Lys	Lys	Vul	Arg	Phie	MET	Ala	Tyr	450
AAT	GGC	CCT	CAG	CGG	ATT	GGT	AGG	AAC	TAC	AAA	AAA	CTC	CCA	TTT	ATC	CCA	TAC	
Thr	Asp	Glu	Thr	Phe	Lys	Thr	Arg	Glu	Ala	Ile	Gln	His	Glu	Ser	Gly	Ile	Leu	468
ACA	GAT	GAA	ACC	TTT	AAC	ACT	CGT	GAA	GCT	ATT	CAG	CAT	TCA	CAA	ATC	TTG		
Gly	Pro	Ileu	Leu	Tyr	Gly	Glu	Val	Gly	Asp	Thr	Leu	Leu	Ile	Ile	Phe	Lys	Asn	486
CGA	CCT	TTA	CTT	TAT	GGG	GAA	GTT	CAA	GAC	ACA	CTG	TTG	ATT	ATA	TTT	AGC	AAT	
Gln	Ala	Ser	Arg	Pro	Tyr	Asn	Ile	Tyr	Pro	His	Gly	Ile	Thr	Asp	Val	GTC	Pro	504
CAA	CCA	ACC	ACA	CCA	TAT	AAC	ATC	TAC	CCT	CAC	CAA	ATC	GAT	GTC	CGT	CCT	CCT	
Leu	Tyr	Ser	Arg	Arg	Leu	Pro	Lys	Gly	Val	Lys	His	Leu	Lys	Asp	Phe	Pro	Ile	522
TTG	TAT	TCA	AGG	AGA	TTA	CCA	AAA	GGT	GTA	AAA	CAT	TTG	AGC	GAT	TTT	CCA	ATT	
Leu	Pro	Gly	Glu	Ile	Phe	Lys	Tyr	Lys	Trp	Thr	Val	Thi	Val	Glu	Asp	Gly	Pro	540
CTG	CCA	CGA	GAA	ATA	TTC	AAA	TAT	AAA	ACA	ACA	GTC	ACT	GTA	GAA	GAT	GGG	CCA	
Thr	Lys	Ser	Asp	Pro	Arg	Cys	Leu	Thr	Arg	Tyr	TAC	TAC	Ser	Ser	Phe	Val	Asn	558
ACT	AAA	TCA	GAT	CCT	CGG	TGC	CTG	ACC	CGC	TAT	TAC	TCT	ACT	TTC	GTT	AAT	MET	
Glu	Arg	Asp	Leu	Ala	Ser	Gly	Leu	Ile	Gly	Pro	Ileu	CTC	ATC	Ile	Cys	Tyr	Lys	576
CAG	AGA	GAT	CTA	GCT	TCA	CGA	CTC	ATT	GGC	CCT	CTC	CTC	ATC	TCC	TAC	CAA	CAA	
Ser	Val	Asp	Gln	Arg	Gly	Asn	Gln	Ile	MET	Ser	Asp	Lys	Arg	Asn	Val	Ile	Leu	594
TCI	CTA	GAT	CAA	ACA	CGA	AAC	CAG	ATA	ATC	TCA	GAC	AGC	AAT	GTC	ATC	CTG		
Phe	Ser	Val	Phe	Asp	Gln	Asn	Arg	Ser	Trp	Tyr	Leu	CTC	ACA	Glu	Asn	Ile	Gln	612
TTT	TCT	CTA	TTT	GAT	CAG	AAC	CGA	ACC	TCC	TAC	TAC	TAC	ATA	ATA	ATA	CAA	CCC	
Phe	Leu	Pro	Asn	Pro	Ala	Cly	Val	Gln	Leu	Glu	Asp	Pro	GCA	Glu	Phe	Gln	Ala	630
TTT	CTC	CCC	AAT	CCA	CCT	CGA	GTC	CAG	CTT	CAC	GAT	CCA	CAC	TTC	CAA	CCC	TCC	
Asn	Ile	MET	His	Ser	Ile	Asn	Cly	Tyr	Val	Phe	Asp	Ser	ACT	Leu	Gln	Leu	Ser	648
AAC	ATC	ATC	CAC	ACC	ATC	AAT	GGC	XAT	CTT	TTT	GAT	ACT	TTT	CAG	TTC	TTC	CTA	

169

212

TABLE 1, continued

Cys	Leu	His	Glu	Val	Ala	Tyr	Trp	Tyr	Ile	Leu	Ser	Ile	Cly	Ala	Gln	Thr	Asp	666
TGT	TTC	CAT	CAG	CTG	CCA	TAC	TCG	TAC	ATT	CTA	ACC	ATT	CGA	CCA	CAG	ACT	CAC	
Phe	Leu	Ser	Val	Phe	Phe	Ser	Gly	Tyr	Thr	Phe	Lys	His	Lys	MET	Val	Tyr	Glu	684
TTC	CTT	TCT	CTC	TTC	TTC	TCT	CGA	TAT	ACC	TTC	AAA	CAC	AAA	ATG	GTC	TAT	CAA	
Asp	Thr	Leu	Ihr	Leu	Phe	Pro	Phe	Ser	Gly	Glu	Thr	Val	Phe	MET	Ser	MET	Glu	702
GAC	ACA	CTC	ACC	CTA	TTC	CCA	TTC	TCA	CGA	CAA	ACT	GTC	TTC	ATG	TCG	ATG	CAA	
Asn	Pro	Gly	Leu	Trp	Ile	Leu	Gly	Cys	His	Asn	Ser	Asp	Phe	Arg	Asn	Arg	Gly	720
AAC	CCA	GGT	CTA	TCG	ATT	CTG	GGG	TCC	CAC	AAC	TCA	CAC	TTT	CCG	AAC	AGA	CCC	
MET	Thr	Ala	Leu	Leu	Lys	Val	Ser	Ser	Cys	Asp	Lys	Asn	Thr	Cly	Asp	Tyr	TAC	738
ATG	ACC	CCC	TTA	CTG	AAG	GTT	TCT	ACT	TGT	GAC	AAG	AAC	ACT	GGT	GAT	TAT	TAC	
Glu	Asp	Ser	Tyr	Glu	Asp	Ile	Ser	Ala	Tyr	Leu	Leu	Ser	Lys	Asn	Asn	Ala	Ile	756
CAG	GAC	ACT	TAT	GAA	CAT	ATT	TCA	GCA	TAC	TTC	CTG	AGT	AAA	AAC	ATT	CCC	ATT	
Glu	Pro	Arg	Ser	Phe	Ser	Gln	Asn	Ser	Arg	His	Pro	Ser	The	Arg	Gln	Lys	Gln	774
CAA	CCA	ACA	ACC	TTC	TCC	CAG	AAT	TCA	AGA	CAC	CCT	ACC	ACT	ACC	CAA	AAC	CAA	
Phe	Asn	Ala	Thr	Thr	Ile	Pro	Glu	Asn	Asp	Ile	Glu	Lys	Thr	Asp	Pro	Trp	Phe	792
TIT	AAT	GCC	ACC	ACA	ATT	CCA	CAA	AAT	GAC	ATA	CAG	AAG	ACT	CAC	CCT	TGG	TTT	
Ala	His	Arg	Thr	Pro	MET	Pro	Lys	Ile	Gln	Asn	Val	Ser	Ser	Ser	Asp	Leu	Leu	810
GCA	CAC	ACA	ACA	CCT	ATG	CCT	AAA	ATA	CAA	AAT	GTC	TCC	ACT	GAT	GAT	TTG	TTC	
MET	Leu	Leu	Arg	Gln	Ser	Pro	Thr	Pro	His	Gly	Leu	Ser	Leu	Ser	Asp	Leu	Gln	828
ATG	CTC	TTG	CGA	CAG	ACT	CCT	ACT	CCA	CAT	GGG	CTA	TCC	TTA	TCT	GAI	CTC	CAA	
Glu	Ala	Lys	Tyr	Glu	Thr	Phe	Ser	Asp	Asp	Pro	Ser	Pro	Gly	Ala	Ile	Asp	Ser	846
CAA	GCC	AAA	TAT	GAG	ACT	TTT	TCT	GAT	GAT	CCA	TCA	CCT	CCA	ATA	CAC	ACT	GGG	
Asn	Asn	Ser	Leu	Ser	Glu	MET	Thr	His	Phe	Arg	Pro	Cln	Leu	His	Ser	Cly		864
AAT	AAC	ACC	CTG	CTC	ATA	TAC	ACA	CAC	TTC	ACC	CCA	CTC	CAT	CAC	ACT	CGG		
Asp	MET	Val	Phe	Ihr	Pro	Glu	Ser	Gly	Leu	Cln	Leu	ATG	Asn	Glu	Lys	Leu		882
GAC	ATG	GTA	TTT	ACC	CCT	GAG	TCA	CCC	CAA	CTC	TTA	ACA	TTA	AAT	GAG	AAA	CTG	
Gly	Thr	Ihr	Ala	Ala	Ihr	Glu	Leu	Lys	Lys	Leu	Asp	The	Lys	Val	Ser	Thr	ACA	900
GGG	ACA	ACT	CCA	GCA	ACA	GAG	TTC	AAG	AAA	CTT	GAT	TTC	AAA	TCT	ACT	ACA		
Ser	Asn	Asn	Leu	Ile	Ser	Thr	Ile	Pro	Ser	Asp	Asn	Leu	Ala	Ala	Gly	Thr	Asp	918
TCA	AAT	AAT	CTG	ATT	TCA	ACA	ATT	CCA	TCA	GAC	ATT	TTG	GCA	CCA	GCT	ACT	CAT	
Asn	Thr	Ser	Ser	Leu	Gly	Pro	Fro	Ser	MET	Pro	Val	His	Tyr	Asp	Ser	Cln	Leu	936
AAT	ACA	AGT	TCC	TTA	CGA	CCC	CCA	ACT	ATG	CCA	CTT	CAT	TAT	GAT	ACT	CAA	TTA	
Asp	Thr	Ihr	Ihr	Leu	Phe	Gly	Lys	Lys	Ser	Ser	Pro	Ile	Thr	Gly	Cly	CCA	954	
GAT	TCC	ACT	CTA	TTT	GGC	AAV	AAC	TCA	TCT	CCC	CTT	ACT	GAC	TCT	GGT	CCA	CCT	
Leu	Ser	Leu	Ser	Glu	Clu	Ala	Asn	Asn	Asp	Ser	Lys	Leu	Leu	Clu	Ser	Gly	MET	972
CTG	ACC	TTC	ACT	CAA	CAA	ATT	ATT	AAT	GAT	TCA	AAG	TTG	TTA	CAA	TCA	CTA	ATC	
Asn	Ser	Gln	Glu	Ser	Ser	Tcp	Gly	Lys	Aen	Aen	Val	Ser	TCA	ACA	Glu	Ser	Arg	990
AAT	ACC	CAA	CAA	AGT	TCA	TCC	CGA	AAA	AAT	CTA	CTG	TCA	ACA	AGT	CGT	AGC	ACC	

176

273

TABLE 1, continued

Leu	Pho	Lys	Gly	Lys	Arg	Ala	His	Gly	Pro	Ala	Leu	Thr	Lys	Asp	Asn	Ala	1,008	
TTA	TTT	AAA	CGG	AAA	ACA	CCT	CAT	CCA	CCT	CCT	TTG	ACT	AAA	CAT	AAT	CCC		
Leu	Phe	Lys	Val	Ser	Ile	Ser	Leu	Leu	Lys	Thr	Ala	Lys	The	Ser	Asn	Ser	1,026	
TTA	TTC	AAA	GTT	ACC	ATC	TCT	TTC	TTA	AAC	ACA	AAC	AAA	ACT	TCC	AAT	TCA		
Ala	Thr	Asn	Arg	Lys	Thr	His	Ile	Asp	Gly	Fro	Ser	Leu	Ile	Glu	Asn	Ser	1,044	
CCA	ACT	AAT	AGA	-AAG	ACT	CAC	ATT	GAT	CCC	CCA	TCA	TTA	TTA	ATT	GAG	AAT	AGT	
Pro	Ser	Val	Trp	Gln	Asn	Ile	Leu	Glu	Ser	Asp	Thr	Glu	Phe	Lys	Lys	Val	Thr	1,062
CCA	TCA	GTC	TGG	CAA	AAT	ATA	TTA	CAA	AGT	GAC	ACT	CAG	TTT	AAA	AAA	CTG	ACA	
Pro	Leu	Ile	His	Asp	Arg	MET	Leu	MET	Asp	Lys	Asn	Ala	Thr	Ala	Leu	Arg	Leu	1,080
CCT	TTG	ATT	CAT	GAC	ACA	AIG	CTT	ATG	GAC	AAA	AAT	GCT	ACA	GCT	TTC	AGG	CTA	
Asn	His	MET	Ser	Asn	Lys	Thr	Thr	Ser	Ser	Lys	Asn	MET	Glu	MET	Val	Gln	Gln	1,098
AAT	CAT	ATG	TCA	AAT	AAA	ACT	ACT	TCA	TCA	AAA	ACC	ATG	CAA	ATG	CTC	CAA	CAG	
Lys	Lys	Glu	Gly	Pro	Ile	Pro	Pro	Asp	Ala	Gln	Asn	Pro	Asp	MET	Ser	Phe	Phe	1,116
AAA	AAA	GAG	GGC	CCC	ATT	CCA	CCA	GAT	CCA	CAA	AAT	CCA	GAT	ATG	ICG	TTC	TTT	
Lys	MET	Leu	Phe	Leu	Pro	Glu	Ser	Ala	Arg	Trp	Ile	Gln	Arg	Thr	His	Gly	Lys	1,134
AAG	ATG	CTA	TTC	TTG	CCA	CAA	TCA	CCA	AGG	TGG	ATA	CAA	AGG	ACT	CAT	CGA	AAAG	
Asn	Ser	Leu	Asn	Ser	Cly	Cln	Cly	Pro	Ser	Pro	Lys	Cln	Leu	Val	Ser	Leu	Gly	1,152
AAC	TCT	CTG	AAC	TCT	GGG	CAA	GGC	CCC	AGT	CCA	AAG	CAA	TTA	GTA	TCC	TTA	CGA	
Pro	Glu	Lys	Ser	Val	Clu	Gly	Gln	Asn	Phe	Leu	Ser	CJu	Lys	Asn	Val	Val	Val	1,170
CCA	GAA	AAA	TCT	GTC	CAA	GGT	CAC	AAT	TTC	TTG	TCT	CAG	AAA	AAC	AAA	CTG	CTA	
Val	Gly	Lys	Gly	Glu	Phe	Thr	Lys	Asp	Val	Gly	Leu	Lys	Glu	MET	Val	Phe	Pro	1,188
GTA	CGA	AAG	CGT	GAA	TTT	ACA	AAG	GAC	GTA	CGA	CTC	AAA	GAG	ATG	CTT	TTT	CCA	
Ser	Ser	Arg	Asn	Leu	Phe	Leu	Thr	Asn	Leu	Asp	Asn	Leu	His	Glu	Asn	Asn	Thr	1,206
ACC	ACC	AGA	AAC	CTA	TTT	CTT	ACT	AAC	TTC	GAT	AAT	TTA	CAT	GAA	AAT	AAT	ACA	
His	Asn	Cln	Glu	Lys	Lys	Ile	Gln	Glu	Glu	Ile	Glu	Lys	Lys	Glu	Thr	Leu	Ile	1,224
CAC	AAT	CAA	CAA	AAA	AAA	ATT	CAG	CAA	CAA	ATA	CAA	AAAG	AAAG	CAA	ACA	TTA	ATC	
Gln	Glu	Asn	Val	Val	Leu	Pro	Gln	Ile	His	Thr	Val	Gly	ACT	Gly	Thr	Asn	Phe	1,242
CAA	GAG	AAT	GTA	GTC	TTG	CCT	CAG	ATA	CAT	ACA	CTG	ACT	GGC	ACT	AAG	AAT	TTC	
MET	Lys	Asn	Leu	Phe	Leu	Leu	Ser	Thr	Arg	Gln	Asn	Val	Glu	Gly	Ser	Tyr	Glu	1,260
ATG	AAC	AAC	CTT	TTC	IAA	CTG	ACC	ACT	AGG	CAA	AAT	GTA	CAA	GGT	TCA	TAT	GAC	
Gly	Ala	Tyr	Ala	Pro	Val	Leu	Gln	Asp	Phe	Arg	Ser	Leu	Asn	Asp	Ser	Thr	Asn	1,278
GGC	GCA	TAT	GCT	CCA	CTA	CTT	CAA	GAT	TTT	AGG	TGA	TTA	AAT	GAT	TCA	ACA	AAT	
Arg	Thr	Lys	Lys	His	Thr	Ala	His	Phe	Ser	Lys	Lys	Gly	Glu	Glu	Asn	Leu	1,296	
AGA	ACA	AAC	AAA	CAC	ACA	GCT	CAT	TTC	TCA	AAA	AAA	GGG	CAG	CAA	AAC	TTG		
Glu	Gly	Leu	Gly	Asn	Cln	Thr	Lys	Cln	Ile	Val	Glu	Lys	Tyr	Ala	Cys	Thr	1,314	
GAA	GGC	TTG	CCA	AAT	CAA	ACC	AAG	CAA	ATT	GTA	GAG	AAA	TAT	CCA	TGC	ACC	ACA	
Arg	Ile	Ser	Pro	Asn	Thr	Ser	Cln	Cln	CAG	Asn	AAT	Phe	Val	Thr	Cln	Arg	Arg	1,332
ACC	ATA	TCT	CCT	AAT	ACA	ACC	CAA	CAG	AAT	TTT	CTC	AGC	CAA	CCT	ACT	AAAC	AGA	

171

216

TABLE 1, continued

Ala	Leu	Lys	Gln	Phe	Arg	Leu	Pro	Leu	Glu	Glu	Thr	Glu	Leu	Glu	Lys	Arg	Ile	1,350
CCT	TTC	AAA	CAA	TTC	AGA	CTC	CCA	CTA	CAA	CAA	ACA	CAA	CTT	CAA	AAA	AGC	ATA	
Ile	Val	Asp	Asp	Thr	Ser	Thr	Cln	Trp	Ser	Lys	Asn	MET	Lys	His	Ile	Thr	Pro	1,368
ATT	GTC	GAT	GAC	ACC	TCA	ACC	CAC	TCC	TCC	AAT	AAC	ATG	AAA	CAT	TTG	ACC	CCG	
Ser	Thr	Leu	The	Gln	Ile	Asp	Tyr	Asn	Glu	Lys	Glu	Lys	Gly	Ala	Ile	Thr	Gln	1,386
ACC	ACC	CTC	ACA	CAG	ATA	GAC	TAC	AAT	CAG	AAG	CAG	AAA	GGG	GCC	ATT	ACT	CAC	
Ser	Pro	Leu	Ser	Asp	Cys	Leu	Thr	Arg	Ser	His	Ser	Ile	Pro	Gln	Ala	Asn	Arg	1,404
TCT	CCC	TTA	TCA	GAT	TGC	CTT	ACC	AGG	ACT	CAT	ACC	ATC	CCT	CAA	CCA	AAT	AGA	
Ser	Pro	Leu	Pro	Ile	Ala	Lys	Val	Ser	Ser	Pho	Pro	Ser	Ile	Arg	Pro	Ile	Tyr	1,422
TCT	CCA	TTA	CCC	ATT	GCA	AAG	GTA	TCA	TCA	TTT	CCA	TCT	ATT	AGA	CCT	ATA	TAT	
Leu	Thr	Arg	Val	Leu	Phe	Gln	Asp	Asn	Ser	Ser	His	Leu	Pro	Ala	Ala	Ser	Tyr	1,440
CTG	ACC	AGG	GTC	CTA	TTC	CAA	GAC	AAC	TCT	TCT	CAT	CTT	CCA	CCA	TCT	TAT		
Arg	Lys	Lys	Asp	Ser	Gly	Val	Gln	Glu	Ser	Ser	His	Phe	Leu	Gln	Gly	Ala	Lys	1,458
ACA	AAG	AAA	GAT	TCT	CGG	GTC	CAA	GAA	AGC	ACT	CAT	TTC	TTA	CAA	GGA	CCC	AAA	
Lys	Alan	Asn	Leu	Ser	Leu	Ala	Ile	Leu	Thr	Leu	Glu	MET	Thr	Cly	Asp	Gln	Arg	1,476
AAA	AAT	AAC	CTT	TCT	TTA	GCC	ATT	CTA	ACC	TTC	CAG	ATG	ACT	GUT	GAT	CAA	AGA	
Glu	Val	Gly	Ser	Leu	Gly	Thr	Ser	Ala	Thr	Asn	Ser	Val	Thr	Tyr	Lys	Lys	Val	1,494
GAG	CTT	GGC	TCC	CTG	GGG	ACA	ACT	GCC	ACA	AAT	TCA	CTC	ACA	TAC	AAA	GTG	CTT	
Glu	Asn	Thr	Val	Leu	Pro	Lys	Pro	Asp	Leu	Pro	Lys	Thr	Ser	Gly	Lys	Val	Glu	1,512
CAG	AAC	ACT	GTG	CTC	CCG	AAA	CCA	GAC	TTC	CCC	AAA	ACA	TCT	GGC	ALA	GTG	CAA	
Leu	Leu	Pro	Lys	Val	His	Ile	Tyr	Gln	Lys	Asp	Leu	Phe	Pro	Thr	Glu	Thr	Ser	1,530
TTC	CTT	CCA	AAA	GTG	CAC	ATT	TAT	CAG	AAG	CAC	CTA	TTC	CCT	AGC	GAA	ACT	AGC	
Asn	Gly	Ser	Pro	Gly	His	Leu	Asp	Leu	Val	Glu	Gly	Ser	Leu	Gln	Gly	GCA	Thr	1,548
AAT	CGG	TCT	CCT	GGC	CAT	CTG	GAT	CTC	GTC	CAA	GGG	ACC	CTT	GTT	CAG	GCA	ACA	
Glu	Gly	Ala	Ile	Lys	Trp	Asn	Glu	Ala	Asn	Arg	Pro	Gly	Lys	Val	Pro	Phe	Leu	1,566
GAG	CGA	CGG	ATT	AAG	TGG	AAT	CAA	CCA	AAC	ACA	CCT	CGA	AAA	CTT	CCC	TTT	CTG	
Arg	Val	Ala	Thr	Glu	Ser	Ser	Ala	Lys	Thr	Pro	Ser	Lys	Leu	Leu	Gly	Pro	Leu	1,584
ACA	GTA	CCA	ACA	GAA	AGC	TCT	GCA	AAG	ACT	CCC	TCC	AAC	CTA	ITG	GAT	CCT	CTT	
Ala	Trp	Asp	Asn	His	Tyr	Gly	Thr	Gln	Ile	Pro	Lys	Glu	Clu	Trp	Lys	Ser	Gln	1,602
CCT	TGG	GAT	AAC	CAC	TAT	GGT	ACT	CAG	ATA	CCA	AAA	GAA	GAG	TTG	CAA	TCC	CAA	
Glu	Lys	Ser	Pro	Glu	Lys	Thr	Ala	Phe	Lys	Lys	Asp	The	Ile	Leu	Ser	TCC	Leu	1,520
CAC	AAG	TCA	CCA	GAA	AAA	ACA	GCT	TTT	AGC	AAA	AAG	GAT	ACC	ATT	ITG	TCC	TIC	
Asn	Ala	Cys	Clu	Ser	Asn	His	Ala	Ile	Ala	Ala	Ile	Asn	Glu	Gly	Gln	Asn	Lys	1,638
AAC	CCT	TGT	CAA	AGC	AAT	CAT	GCA	ATA	GCA	CCA	ATA	ATA	ATA	GAG	CAA	AAT	AAA	
Pro	Glu	Ile	Glu	Val	Thr	Trp	Ala	Lys	Gln	Cly	Arg	The	Glu	Arg	Leu	Cys	Ser	1,656
CCC	GAA	ATA	CAA	GTC	ACC	TGG	CCA	AAC	CAA	GCA	GCT	AGC	ACT	GAA	AGG	CTG	TCT	
Gln	Asn	Pro	Pro	Vnl	Ile	Lys	Arg	His	Gln	Arg	Glu	Ile	Thr	Arg	The	Ile	Leu	1,674
CAA	AAC	CCA	CCA	GTC	TTC	AAA	CCC	CAT	CAA	CCC	ATA	ACT	CGT	ACT	ACT	CTT		

172

215

TABLE 1, continued

Cln	Ser	Asp	Cln	Glu	Glu	Ile	Asp	Tyr	Asp	Asp	Thr	Ile	Ser	Val	Glu	MET	Lys	1,692
CAG	TCG	CAT	CAA	GAG	CAA	ATT	WAC	TAT	CAT	CAT	ACC	ATA	TCA	GTT	GAA	ATG	AAG	
Lys	Glu	Asp	The	Asp	Ile	Tyr	Asp	Glu	Asp	Glu	Asn	Cln	Ser	Pro	Arg	Ser	Phe	1,710
AAC	CAA	CAT	TTT	GAC	ATT	TAT	CAT	GAG	CAT	GAA	AAT	CAC	ACC	CCC	CCG	ACC	TTT	
Cln	Lys	Lys	The	Arg	His	Tyr	Phe	Ile	Ala	Ala	Val	Cln	Arg	Leu	Trp	Asp	Tyr	1,728
CAA	AAG	AAA	ACA	CCA	CAC	TAT	TTT	ATT	GCT	GCA	GTC	CAG	AGC	CTC	TGG	CAT	TAT	
Gly	MET	Ser	Ser	Ser	Pro	His	Val	Leu	Arg	Asn	Arg	Ala	Cln	Ser	Gly	Ser	Val	1,746
GGG	ATG	ACT	ACC	TCC	CCA	CAT	GTT	CTA	AGA	AAC	AGC	CAC	ACT	GTG	GGC	ACT	GTC	
Pro	Cln	Phe	Lys	Lys	Val	Val	Phe	Cln	Glu	Phe	Thr	Asp	Gly	Ser	Phe	Thr	Cln	1,764
CCT	CAG	TTC	AAG	AAA	GTT	CTT	TTC	CAG	CAA	TTT	ACT	GAT	CCC	TCC	TTT	ACT	CAG	
Pro	Leu	Tyr	Arg	Gly	Glu	Leu	Asn	Glu	His	Leu	Gly	Leu	Leu	Gly	Pro	Tyr	Ile	1,782
CCC	TTA	TAC	CCT	CGA	CAA	CTA	AAT	GAA	CAT	TTG	GGA	CTC	CTG	GGG	CCA	TAT	ATA	
Arg	Ala	Glu	Val	Glu	Asp	Asn	Ile	MET	Val	Thr	Phe	Arg	Asn	Cln	Ala	Ser	Arg	1,800
AGA	CCA	CAA	GTT	GAA	CAT	AAT	ATC	ATG	GTA	ACT	TTC	ACA	AAT	CAG	CCC	TCT	CGT	
Pro	Tyr	Ser	Phe	Tyr	Ser	Ser	Leu	Ile	Ser	Tyr	Glu	Glu	Asp	Gln	Arg	Cln	Gly	1,818
CCC	TAT	TCC	TTC	TAT	TCT	AGC	CTT	ATT	TCT	TAT	CAG	GAA	GAT	CAG	AGG	CAA	GGG	
Ala	Glu	Pro	Arg	Lys	Asn	Phe	Val	Lys	Pro	Asn	Glu	Thr	Lys	Thr	Phe	Trp	TGG	1,836
CCA	GAA	CCT	AGA	AAA	AAC	TTT	GTC	AAG	CCT	AAT	GAA	ACC	AAA	ACT	TAC	TTT	TGG	
Lys	Val	Cln	His	His	MET	Ala	Pro	Thr	Lys	Asp	Glu	Phe	Asp	cys	Lys	Kia	Trp	1,854
AAA	CTG	CAA	CAT	CAT	ATG	GCA	CCC	ACT	AAA	GAT	TTC	GAC	TTT	TCC	AAA	CCC	TGC	
Ala	Tyr	Phe	Ser	Asp	Val	Asp	Leu	Glu	Lys	Asp	Val	His	Ser	Gly	Leu	Ile	Gly	1,872
GCT	TAT	TTC	TCT	GAT	GTT	GAC	CIG	CAA	AAA	CAT	GTC	CAC	TCA	GGC	CTG	ATT	CGA	
Pro	Leu	Leu	Val	Cys	His	Thr	Asn	Thr	Leu	Asn	Pro	Ala	Gly	Arg	Cln	Val	CTG	1,890
CCC	CTT	CTG	CTC	TGC	CAC	ACT	AAA	ACA	CTG	AAC	CCT	CAT	GGG	AGA	CAA	CTG		
Thr	Val	Cln	Glu	Phe	Ala	Leu	Phe	Thr	Ile	Phe	Asp	Glu	Thr	Lys	Ser	Trp	TGG	1,908
ACA	GIA	CAG	GAA	TTT	GCT	CTG	TTT	TTC	ACC	ATC	TTT	GAT	GAG	ACC	AAA	AGC	TGG	
Thy	Phe	Thr	Glu	Asn	MET	Glu	Arg	Asn	Cys	Arg	Ala	Pro	Cys	Asn	Ile	Gln	MET	1,926
TAC	TTC	ACT	CAA	AAT	ATG	GAA	AGA	AAC	TCC	ACC	GCT	CCC	TCC	AAT	ATC	CAG	ATC	
Glu	Asp	Pro	Thr	Phe	Lys	Glu	Asn	Thr	Arg	Phe	His	Ala	Ile	Asn	Gly	Tyr	Ile	1,944
CAA	CAT	CCC	ACT	TTT	AAA	CAG	AAT	TAT	CCG	TTC	CAT	ATC	ATC	CCC	GGC	TAC	ATA	
MET	Asp	Thr	Leu	Pro	Gly	Leu	Val	MET	Ala	Gln	Asp	Gln	Arg	Ile	Arg	Trp	TAT	1,962
ATG	CAT	ACA	CTA	CCT	GGC	TTA	GTA	ATG	GCT	CAG	GAT	CAA	AGG	ATT	CCA	TGG	TAT	
Leu	Leu	Ser	MET	Gly	Ser	Asn	Glu	Asn	Ile	His	Ser	Ile	His	Phe	Ser	Cly	His	1,980
CTG	CTC	ACC	ATG	CCC	ACC	AAT	CAA	AAC	ATC	CAT	TCT	ATT	CAT	TTC	ACT	CAA	CAT	
Val	Phe	Thr	Val	Arg	Lys	Lys	Glu	Glu	Tyr	Lys	MET	Ala	Ieu	Tyr	Asn	Ieu	TAT	1,998
CTG	TTC	ACT	GTA	CGA	AAA	AAA	CAA	GAG	TAT	AAA	ATC	GCA	CTG	TAC	AAAT	CTC		
Pro	Gly	Val	Phe	Glu	Thr	Val	Glu	Glu	MET	Leu	Pro	Ser	Lys	Ala	Gly	Ile	TGG	2,016
CCA	GGT	CTT	TTT	CAC	ACA	GTC	CAA	ATC	TTA	CCA	TCC	AAA	GCT	ATT	TGG	AGC		

173

216

TABLE 1, continued

Val	Glu	Cys	Leu	Ile	Gly	Glu	His	Leu	His	Ala	Gly	MET	Ser	Thr	Leu	Phe	Leu	2,034
GTC	GAA	TCC	CTT	ATT	CCC	GAC	CAT	GTC	CAT	CCT	GGG	ATG	AGC	ACA	TTT	TTT	CTG	
Val	Tyr	Ser	Asn	Lys	Cys	Gln	Thr	Pro	Leu	Gly	MET	Ala	Ser	Gly	His	Ile	Arg	2,052
GTC	TAC	ACC	AAT	AAG	TGT	CAG	ACT	CCC	CTG	CGA	ATG	GCT	TCT	GGA	CAC	ATT	AGA	
Asp	Phe	Gln	Ile	Ihr	Ala	Ser	Gly	Gln	Tyr	Gly	Cln	Trp	Ala	Pro	Lys	Leu	Ala	2,070
GAT	TTT	CAG	ATT	ACA	GCT	TCA	CCA	CAA	TAT	GCA	CAG	TGC	CCC	CCA	AAC	CTG	CCC	
Arg	Leu	His	Tyr	Ser	Gly	Ser	Ile	Asn	Ala	Trp	Ser	Thr	Lys	Glu	Pro	Phe	Ser	2,088
ACA	CTT	CAT	TAT	TCC	GCA	TCA	ATC	AAT	GCC	TCG	ACC	AGC	ACC	AAG	CAG	TTT	TCT	
Trp	Ile	Lys	Val	Asp	Leu	Leu	Ala	Pro	MET	Ile	Ile	His	Gly	Ile	Lys	Thr	Gln	2,106
TCG	ATC	AAG	GTG	CAT	CTG	TTG	CCA	CCA	ATG	ATT	CAC	GGC	ATC	AAG	ACC	CAC		
Gly	Ala	Arg	Gln	Lys	Phe	Ser	Ser	Leu	Tyr	Ile	Ser	Gln	Phe	Ile	Ile	MET	Tyr	2,124
GGT	GCC	CGT	CAG	AAG	TTC	TCC	ACC	CTC	TAC	ATC	TCT	CAG	TTT	ATC	ATC	ATG	TAT	
Ser	Leu	Asp	Gly	Lys	Lys	Trp	Gln	Thr	Tyr	Arg	Gly	Asn	Ser	Thr	Gly	Thr	Leu	2,142
AGT	CTT	GAT	GGG	AAG	AAG	TGG	CAG	ACT	TAT	CGA	CCA	AAT	TCC	ACT	CCA	ACC	TTA	
MET	Val	Phe	Phe	Gly	Asn	Val	Asp	Ser	Ser	Gly	Ile	Lys	His	Asn	Ile	Phe	Asn	2,160
ATG	CTC	TTC	TTT	GGC	AAT	CTG	CAT	TCA	TCT	CGG	ATA	AAA	CAC	AAT	ATT	TTT	AAC	
Pro	Pro	Ile	Ile	Ala	Arg	Tyr	Ile	Arg	Leu	His	Pro	Thr	His	Tyr	Ser	Ile	Arg	2,178
CCT	CCA	ATT	ATT	CCT	CGA	TAC	ATC	CGT	TTC	CAC	CCA	ACT	CAT	TAT	ACC	CCC		
Ser	Ihr	Leu	Arg	MET	Glu	Leu	MET	Gly	Cys	Asp	Leu	Asn	Ser	Cys	Ser	MET	Pro	2,196
AGC	ACT	CTT	CGC	ATG	GAC	TTC	ATG	CCC	TGT	GAT	TTA	AAT	ACT	TCC	ACC	ATG	CCA	
Leu	Gly	MET	Glu	Ser	Lys	Ala	Ile	Ser	Asp	Ala	Cln	Ile	Thr	Ala	Ser	Tyc	TAC	2,214
TTC	GCA	ATG	GAC	ACT	AAA	GCA	ATA	TCA	GAT	CCA	CAG	ATT	ACT	GCT	TCA	ACC	TAC	
Phe	Thr	Asn	MET	Phe	Ala	Ihr	Trp	Ser	Pro	Ser	Lys	Ala	Arg	Leu	His	Leu	Cln	2,232
ITT	ACC	AAI	ATG	TTT	GCC	ACC	TGG	TCT	CCT	TCA	AAA	GCT	AAA	CGA	CTT	CAC	CAA	
Gly	Arg	Ser	Asn	Ala	Trp	Arg	Pro	Gln	Val	Asn	Asn	Pro	Lys	Glu	Trp	Leu	Cln	2,250
GGC	AGG	ACT	AAT	CCC	TGG	AGA	CCT	CAG	GTC	AAT	AAT	CCA	AAA	GAC	TGG	CTG	CAA	
Val	Asp	Phe	Gln	Lys	Thr	MPT	Lys	Val	Ihr	Gly	Val	CTA	Thr	Thr	Gln	Gly	Val	2,268
GTC	GAC	ITC	CAG	AAG	ACA	ATG	AAA	CTC	ACA	AAA	CTA	CTG	ACT	ACT	CAG	CTA	AAA	
Ser	Leu	Leu	Thr	Ser	MET	Tyr	Val	Lys	Glu	Phe	Leu	Ile	Ser	Ser	Ser	Cln	Asp	2,286
TCT	CTC	CTT	ACC	ACC	ATG	IAT	CTG	AAG	GAG	TTC	CTC	ATC	TCC	ACC	ACT	CAA	GAT	
Gly	His	Gln	Trp	Thr	Leu	Phe	Phe	Gln	Asn	Gly	Lys	Vnl	Lys	Val	Phe	Gln	Gly	2,304
GGC	CAT	CAC	TCG	ACT	CTC	TTT	TTT	CAG	AAT	CCC	AAA	CTA	AAC	CTT	TTT	CGA	CGA	
Asn	Gln	Asp	Ser	Phe	Ihr	Pro	Val	Val	Asn	Ser	Lys	Vnl	Lys	Val	Phe	Gly	CGA	2,322
AAT	CAA	GAC	TCC	TTC	ACA	CCT	CTG	CTG	AAC	TCT	CTA	Asp	CCA	Pro	CTG	TTA		
Arg	Tyr	Leu	Arg	Ile	His	Pro	Gln	Ser	Ihr	Val	His	Gln	Ile	Ala	Leu	Arg	MET	2,340
CCC	TAC	CTT	CGA	ATT	CAC	CCC	CAG	AGT	TCC	CTG	CAC	CTG	ATT	CCC	CTG	AGC	ATG	
Clu	Val	Leu	Gly	Cys	Glu	Ala	Gln	Asp	Leu	Tyr	End	TAC	TGA	GGGTGGCCACTGCATUCCACCTGUCACTG			2,352	
CAG	GTT	CTG	GGC	TCC	GAC	CCA	CAG	GAC	CTG	TAC	TGA							

CCGTACCTCTCCCTCAGCTCCAGGCCATGTCCTCCCTGGCTTGCTTCTACCTTCTGCTAAATCTACCACTACCTCC

AAGCCTCTGAATTAACATCATCACTCTCCATTCTTCTCCCCCCCCAGGAGGGCTCCATCCATTAACTTAACCTTACCTATT

TTCTGCCACCTGCTCCCAGA

174

217

1000 followed by the amino acid sequence of Asp-1582 to Arg--
1708. That compound thus comprises the polypeptide sequence
of Ala-20 to Pro-1000 covalently linked by a peptide bond to
amino acids Asp-1582 to Tyr-2351. Another exemplary compound
5 contains a region X comprising the amino acid sequence Ser-760
to Thr-778 followed by the sequence Pro-1659 to Arg-1708.
That compound thus comprises the polypeptide sequence Ala-20
to Thr-778 covalently linked by a peptide bond to the sequence
Pro-1659 through Tyr-2351. Still another exemplary compound
10 contains a region X comprising the amino acid sequence Ser-760
to Thr-778 followed by the sequence Glu-1694 to Arg-1708.
That compound thus comprises the polypeptide sequence Ala-20
to Thr-778 covalently linked by a peptide bond to amino acids
Glu-1694 through Tyr-2351.

15 These exemplary compounds are depicted schematically in
Table 2.

The amino acid sequence represented by X should be selected
so that it does not substantially reduce the procoagulant
20 activity of the molecule, which activity can be conveniently
assayed by conventional methods. Compound (2) of Table 2 is a
presently preferred embodiment.

The procoagulant protein may be produced by appropriate host
25 cells transformed by factor VIII:C DNA which has been specifically
altered by use of any of a variety of site-specific mutagenesis
techniques which will be familiar to those of ordinary
skill in the art of recombinant DNA.

30 The starting materials may be a DNA sequence which codes for
the complete factor VIII:C molecule, e.g., the complete human
factor VIII:C as shown in Table 1, a truncated version of that
sequence, or it may comprise segments of that DNA sequence, so
long as the starting materials contain at least sufficient DNA
35 to code for the amino acid sequences of the desired polypeptide.

175

218

TABLE 2: EXEMPLARY COMPOUNDS A-X-B

<u>Compound</u>	<u>Amino Acid Sequence</u>	<u>X</u>	<u>Delc</u>
(human factor VIII:c)	(Ala ₂₀ → Tyr ₂₃₅₁)	(Ser ₇₆₀ → Arg ₁₇₀₈)	
1	(Ala ₂₀ → Pro ₁₀₀₀) - (Asp ₁₅₈₂ → Tyr ₂₃₅₁)	(Ser ₇₆₀ → Pro ₁₀₀₀) - (Asp ₁₅₈₂ → Arg ₁₇₀₈)	
2	(Ala ₂₀ → Thr ₇₇₈) - (Pro ₁₆₅₉ → Tyr ₂₃₅₁)	(Ser ₇₆₀ → Thr ₇₇₈) - (Pro ₁₆₅₉ → Arg ₁₇₀₈)	
3	(Ala ₂₀ → Thr ₇₇₈) - (Glu ₁₆₉₄ → Tyr ₂₃₅₁)	(Ser ₇₆₀ → Thr ₇₇₈) - (Glu ₁₆₉₄ → Arg ₁₇₀₈)	

A and B are as defined, supra; "-" represents a peptide bond; "→" indicates a polypeptide sequence inclusive of the specified amino acids; amino acid numbering corresponds to the numbering of the sequence depicted in Table I; and "deletion" indicates the number of amino acids deleted relative to human factor VIII:c.

176

269

The procoagulant proteins of the present invention, in addition to lacking a substantial amino acid segment of human factor VIII:C, also have fewer potential N-glycosylation sites than human factor VIII. Preferably, at least one N-glycosylation site 5 has been deleted. More preferably, 18 of the 25 potential N-glycosylation sites are not in the molecule. In still more preferred embodiments, up to 19 of the 25 potential N-glycosylation sites are removed. While not wishing to be bound by theory, it is presently believed that the antibodies to factor 10 VIII:C which are directed to antigenic determinants contained in the protein segment deleted in accordance with this invention, i.e., in the amino acid segment itself or in the carbohydrate portion of the glycosylated protein, will not neutralize the procoagulant proteins of the present invention. Moreover, 15 the fact that the procoagulants of the present invention lack many of the sites for non-human glycosylation by the non-human mammalian or other cells used to produce the proteins is also believed to reduce the antigenicity of that protein, and lessen the likelihood of developing antibodies to the procoagulants. 20 This may enable facilitating the treatment of patients in need of procoagulant therapy.

I contemplate that my compounds can be produced by recombinant DNA techniques at a much lower cost than is possible for production of human factor VIII. The host organisms should more efficiently process and express the substantially simpler molecules of this invention.

The compounds of this invention can be formulated into pharmaceutically acceptable preparations with parenterally acceptable vehicles and excipients in accordance with procedures known 30 in the art.

The pharmaceutical preparations of this invention, suitable for 35 parenteral administration, may conveniently comprise a sterile lyophilized preparation of the protein which may be reconsti-

tuted by addition of sterile solution to produce solutions preferably isotonic with the blood of the recipient. The preparation may be presented in unit or multi-dose containers, e.g. in sealed ampoules or vials. Their use would be analogous 5 to that of human factor VIII, appropriately adjusted for potency.

One method by which these proteins can be expressed is by use of DNA which is prepared by cutting a full-length factor VIII:C DNA with the appropriate restriction enzymes to remove 10 a portion of the DNA sequence that codes for amino acids 760 to 1708 of human factor VIII:C. The cut DNA is then ligated with an oligonucleotide that resects the cut DNA and maintains the correct translational reading frame.

15 Preparation of the cDNA has been set forth in detail in U.S. Patent Applications Serial Nos. 546,650 and 644,086, supra. A pSP64 recombinant clone containing the nucleotide sequence depicted in Table 1, designated as pSP64-VIII, is on deposit at the American Type Culture Collection under Accession Number 20 ATCC 39812.

Restriction endonucleases are used to obtain cleavage of the human factor VIII:C cDNA, hereinafter the DNA source sequence, at appropriate sites in the nucleotide sequence. Unless 25 otherwise noted, restriction endonucleases are utilized under the conditions and in the manner recommended by their commercial suppliers. The restriction endonucleases selected herein are those which will enable one to excise with substantial specificity sequences that code for the portion of the factor 30 VIII:C molecule desired to be excised. BamHI and SacI are particularly useful endonucleases. However, the skilled artisan will be able to utilize other restriction endonucleases chosen by conventional selection methods. The number of nucleotides deleted may vary but care should be taken to 35 insure that the reading frame of the ultimate cDNA sequence will not be affected.

178

-22-

The resulting DNA fragments are then purified using conventional techniques such as those set forth in Maniatis et al., Molecular Cloning, A Laboratory Manual (Cold Spring Harbor Laboratory 1982) the disclosure of which is incorporated herein by reference, and 5 Proc. Natl. Acad. Sci. 76:615-619 (1979). The purified DNA is then ligated to form the sequence encoding the polypeptide of the preferred invention. When necessary or desirable, the ligation may be within an oligonucleotide that resects the cut DNA and maintains the correct translational reading frame 10 using standard ligation conditions. Ligation reactions are carried on as described by Maniatis et al., supra at 2453-6 using the buffer described at page 246 thereof and using a DNA concentration of 1-100 ug/ml, at a temperature of 23°C for blunt ended DNA and 16°C for "sticky ended" DNA. The following 15 double-stranded oligonucleotide is useful when there is BamHI/- SacI deletion such as described infra,

5' P-CATGGACCG-3'
3'-TCGAGTACCTGGCCTAG 5';

20 but other oligonucleotides can be selected by the skilled artisan depending upon the deletions made and reaction conditions.

The DNA sequences encoding the novel procoagulant polypeptides can, in addition to other methods, be derived from the sequence 25 of human factor VIII:C DNA by application of oligonucleotide-mediated deletion mutagenesis, often referred to as "loopout" mutagenesis, as described for example in Morinaga, Y. et al. Biotechnology, 2: 636-639 (1984).

30 The new DNA sequences containing the various deletions can then be introduced into appropriate vectors for expression in mammalian cells. The procoagulant activity produced by the transiently transfected or stably transformed host cells may 35 be measured by using standard assays for blood plasma samples.

The eukaryotic cell expression vectors described herein may be synthesized by techniques well known to those skilled in this art. The components of the vectors such as the bacterial replicons, selection genes, enhancers, promoters, and the like 5 may be obtained from natural sources or synthesized by known procedures. See Kaufman et al., J. Mol. Biol., 159: 51-521 (1982); Kaufman, Proc. Natl. Acad. Sci. 82: 689-693 (1985).

Established cell lines, including transformed cell lines, are 10 suitable as hosts. Normal diploid cells, cell strains derived from in vitro culture of primary tissue, as well as primary explants (including relatively undifferentiated cells such as haematopoietic stem cells) are also suitable. Candidate cells need not be genotypically deficient in the selection gene so 15 long as the selection gene is dominantly acting.

The host cells preferably will be established mammalian cell lines. For stable integration of the vector DNA into chromosomal DNA, and for subsequent amplification of the integrated vector 20 DNA, CHO (Chinese hamster ovary) cells are presently preferred. See U.S. Patent 4,399,216. Alternatively, the vector DNA could include all or parts of the bovine papilloma virus genome (Lusky et al., Cell, 36: 391-401 (1984)) and be carried in cell lines such as C127 mouse cells as a stable episomal 25 element. Other usable mammalian cell lines include HeLa, COS-1 monkey cells, melanoma cell lines such as Bowes cells, mouse L-929 cells, 3T3 lines derived from Swiss, Balb-c or NIH mice, BHK or HaK hamster cells lines and the like.

30 Stable transformants then are screened for expression of the procoagulant product by standard immunological or enzymatic assays. The presence of the DNA encoding the procoagulant proteins may be detected by standard procedures such as Southern blotting. Transient expression of the procoagulant genes 35 during the several days after introduction of the expression

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vector DNA into suitable host cells such as COS-1 monkey cells is measured without selection by enzymatic or immunologic assay of the proteins in the culture medium.

- 5 The invention will be further understood with reference to the following illustrative embodiments, which are purely exemplary, and should not be taken as limiting the true scope of the present invention, as described in the claims.

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EXAMPLE 1

10 ug. of the plasmid pACE, a pSP64 (Promega Biotec, Madison, Wis.) derivative, containing nucleotides 562-7269 of human factor VIII:C cDNA (nucleotide 1 is the A of the ATG initiator methionine codon) was subjected to partial BamHI digestion in 100ul containing 50mM Tris.HCl ph 8.0, 50mM MgCl₂, and 2.4 units BamHI (New England Biolabs) for 30 minutes at 37°C. The reaction was terminated by the addition of EDTA to 20mM and then extracted once with phenol, once with chloroform , ethanol 10 precipitated and pelleted by centrifugation. DNA was redissolved, cleaved to completion in 50ul using 40 units SacI for 1.5 hours at 37°C. DNA was then electrophoresed through a buffered 0.6% agarose gel. An 8.1 kb fragment corresponding to the partial BamHI-SacI fragment of pACE lacking only the 15 sequence corresponding to nucleotides 2992-4774 of the factor VIII:C sequence was purified from the gel using the glass powder technique described in Proc. Nat. Acad. Sci. 76; 615-619 (1979). Purified DNA was ligated with 100 pmoles of the following double-stranded oligonucleotide

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5'P-CATGGACCG-3'
3'-TCGAGTACCTGGCCTAG 5'

using standard ligation conditions. The DNA sequence removed represents the deletion of 584 amino acid sequence beginning 25 with amino acid 998 and continuing through 1581. The oligonucleotide inserted, however, encodes amino acids corresponding to 998-1000. Therefore, the polypeptide encoded contains deletion of 581 amino acids.

30 DNA was then used to transform competent E. coli bacteria, and DNA from several ampicillin resistant transformants was analyzed by restriction mapping to identify a plasmid harboring the desired SacI-BamHI deletion mutant. DNA from this plasmid was digested to completion with KpnI, which cleaves the plasmid 35 uniquely at nucleotide 1816 of the factor VIII:C coding se-

1C 2

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quence. This DNA was ligated with a KpnI DNA fragment containing nucleotides 1-1815 of factor VIII:C DNA and a synthetic SalI site at nucleotides -11 to -5 and then used to transform competent E. coli bacteria.

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Plasmid DNA was isolated and oriented by restriction mapping to identify a plasmid, pBSDK, containing the correct 5' to 3' orientation of the KpnI insert. SalI digestion, which excises the entire polypeptide coding region from the plasmid, was performed 10 and the DNA electrophoresed through a buffered 0.6% agarose gel. The 5.3Kb SalI fragment was purified from the gel as described above. This DNA fragment was ligated with XhoI cut pXMT2 DNA to give rise to plasmid pDGR-2. pXMT2 is a plasmid capable of expressing heterologous genes when introduced into mammalian 15 cells such as the COS-1 African Green Monkey kidney cell line, and is a derivative of the expression vectors described in Kaufman, supra at 689-93. The expression elements are the same as described for plasmid pQ2 except that it contains a deletion of the adenovirus major late promoter extending from 20 -45 to +156 with respect to the transcription start site of the adenovirus major late promoter. mRNA expression in pXMT is driven by the SV40 late promoter. The bacterial replicon, however, has been substituted to render bacteria containing the vector resistant to ampicillin rather than tetracycline. 25 pXMT2 contains a unique Xho I site at a position which allows for expression of inserted cDNA from the SV40 late promoter. This Xho I site is convenient for inserting factor VIII:C cDNA constructs since these are flanked by SalI sites.

30 Restriction mapping of transformants identified a plasmid, pDGR-2, containing the correct 5' to 3' orientation of the polypeptide coding sequence relative to the direction of transcription from the SV40 late promoter. pDGR-2 is on deposit at the American Type Culture Collection under Accession number
35 53100.

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EXAMPLE 2

Other novel procoagulant proteins may be obtained from constructs produced by oligonucleotide mediated deletion mutagenesis, using 5 for example the "loopout" mutagenesis techniques as described in Morinaga et al., supra. The deletion mutagenesis is performed using expression plasmid pDGR-2 or any other appropriate plasmid or bacteriophage vector. Other methods for oligonucleotide mediated mutagenesis employing single stranded DNA produced with 10 M13 vectors and the like are also suitable. See Zoller et al., Nucl. Acids Res. 10: 6487-6500 (1982). For example, these deletions can be produced using the oligonucleotides

(A) 5' AAAAGCAATTAAATGCCACCCACCAAGTCTTGAAACGCCA
15 (B) 5' AAAAGCAATTAAATGCCACCGAAGATTTGACATTTATGA

to cause deletions in factor VIII:C cDNA from nucleotides (A) 2334 to 4974 or (B) 2334 to 5079. The proteins encoded by these constructs contain deletions of (A) 880 and (B) 915 amino acids 20 relative to Factor VIII:C.

The deleted constructs are tested directly, or after subcloning into appropriate expression vectors, in order to determine if the novel proteins possess procoagulant activity. Procoagulant 25 activity was assayed as described in Examples 3 and 4.

EXAMPLE 3

Expression of Procoagulant Molecules in COS Monkey Cells

30 The expression plasmids containing the modified cDNA's prepared as in Examples 1 or 2 and the full-length cDNA, pXMT-VIII, were introduced into COS-1 cells via the DEAE-dextran transfection protocol. Sompayrac and Dana 1981, Proc. Natl. Acad. Sci. 78: 7575-7578. Conditioned media was harvested 48 hours 35 post-transfection and assayed for factor VIII-type activity as described in Toole et. al., 1984, Nature 312:342-347. The

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results of the experiment are summarized in Table 3. Both plasmids containing the modified cDNAs yielded procoagulant activity and, moreover, the activity was greater than that obtained using wild type cDNA. From these data it was concluded
5 that removal of up to 880 amino acids (95,000 daltons) in a defined domain of human factor VIII does not destroy cofactor activity. Furthermore, these abridged procoagulant proteins retain their ability to be activated by thrombin.

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TABLE 3: EXPRESSION OF ABRIDGED FACTOR VIII MOLECULES

	# amino acids deleted	chromogenic activity (mUml^{-1})	Clotek activity -IIa	+IIa (fold)
No DNA	-	0		
pXMT-VIII	-	15:1	-	450
pDGR-2	581	114	250	5750 (23X)
pLA-2	880	162	330	9240 (28X)

The plasmids indicated were transfected into COS cells and 48 hr. post-transfection the conditioned media taken for assay by the Kabi Coatest factor VIII:C method (chromogenic activity) and by the one-stage activated partial thromboplastin time (APTT) coagulation assay (Clotek activity) using factor VIII:C deficient plasma as described (Toole, Nature 1984). For thrombin (IIa) activation, samples were pretreated 1-10 min, with 0.2 units/ml thrombin (IIa) at room temperature. Activation coefficients are provided in parentheses. Activity from media from the wild-type (pXMT-VIII) transfection was too low to directly measure Clotek activity before thrombin activation. From other experiments where the wild type factor VIII activity was concentrated, it was demonstrated to be approximately 30-fold activatable.

EXAMPLE 4

Expression of Procoagulant Molecules in CHO Cells

A) Expression of pDGR-2

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The procoagulant expression vector containing a deletion (relative to the Factor VIII:C cDNA) of 581 amino acids (pDGR-2) was transfected with plasmid pAdD26SV(A) #3 (10 ug pDGR-2:1 ug pAdD26SV(A) #3) by CaPO₄ coprecipitation into CHO DHFR deficient 10 cells (DUKX-B11) and transformants isolated and grown in increasing concentrations of MTX as described by Kaufman et. al., (1985). One transformant designated J1 exhibited the following activities as a function of resistance to increasing concentrations of MTX.

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<u>uM MTX</u>	<u>mUnits/ml/day/10⁶ cells*</u>
0	1.46
0.02	322
0.1	499

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B) Expression of pLA-2

The procoagulant expression vector containing a deletion of 880 amino acids (pLA-2) was introduced into CHO DHFR deficient 25 cells (DUKX-B11, Chasin and Urlaub, PNAS 77: 4216-4220, 1980 by protoplast fusion as described (Sandri-Goldin et. al., Mol. Cell. Biol. 1: 743-752). After fusion, fresh medium containing 100 ug/ml of kanamycin, and 10 ug/ml of each of thymidine, adenosine, deoxyadenosine, penicillin, and streptomycin and 30 10% dialyzed fetal calf serum was added to each plate. The kanamycin was included to prevent the growth of any bacteria which had escaped conversion to protoplasts. Four days later the cells were subcultured 1:15 into alpha-media with 10% dialyzed fetal calf serum, penicillin, and streptomycin, but 35 lacking the nucleosides. Colonies appeared after 10-12 days after subculturing cells into selective media. A group of 8

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transformants were pooled and grown in sequentially increasing concentrations of MTX starting at 0.02 uM with steps to 0.1, 0.2, and 1.0 uM MTX. Results of factor VIII-type activity in cells resistant to increasing concentrations of MTX is shown
 5 below.

<u>uM MTX</u>	<u>mUnits/ml/day/10⁶cells*</u>
0	16
0.02	530
10 0.2	1170
1.0	1890

* Factor VIII activity was determined by the Kabi Coatest factor VIII:C method (chromogenic activity).

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What is claimed is:

1. A protein exhibiting procoagulant activity having the amino acid sequence:

A-X-B

wherein region A represents the polypeptide sequence Ala-20 through Arg-759 substantially as shown in Table 1; region B represents the polypeptide sequence Ser-1709 through Tyr 2351 substantially as shown in Table 1; and region X represents a polypeptide sequence comprising up to 949 amino acids substantially duplicative of sequences of amino acids within the sequence Ser-760 through Arg-1708 of Table 1, wherein the amino terminus of X is covalently bonded through a peptide bond to the carboxy terminus of A, and the carboxy terminus of X is likewise bonded to the amino terminus of B.

2. A protein of claim 1 comprising the amino acid sequence Ala-20 through Pro-1000 followed by Asp-1582 through Tyr-2351 substantially as shown in Table 1 wherein Pro-1000 is covalently bonded by a peptide bond to Asp-1582.
3. A protein of claim 1 comprising the amino acid sequence Ala-20 through Thr-778 followed by Pro-1659 through Tyr-2351, substantially as shown in Table 1, wherein Thr-778 is covalently bonded by a peptide bond to Pro-1659.
4. A protein of claim 1 comprising the amino acid sequence Ala-20 through Thr-778 followed by Glu-1694 through Tyr-2351, substantially as shown in Table 1, wherein Thr-778 is covalently bonded by a peptide bond to Glu-1694.
5. A DNA molecule encoding the protein of claim 1.

189

251

6. A DNA molecule encoding the protein of claim 2.
7. A DNA molecule encoding the protein of claim 3.
8. A DNA molecule encoding the protein of claim 4.
9. A genetically engineered host cell containing, and capable of expressing, a DNA molecule encoding the protein of claim 1.
10. A genetically engineered host cell of claim 9 wherein the host cell is a mammalian, yeast or bacterial cell.
11. A method for producing a protein exhibiting procoagulant properties which comprises culturing a genetically engineered cell of claim 9 under suitable conditions permitting expression of the protein.
12. A pharmaceutical preparation useful for therapeutic treatment of Hemophilia A comprising a sterile preparation of a protein of claim 1 in admixture with a pharmaceutically accepted carrier.
13. A pharmaceutical preparation useful for therapeutic treatment of Hemophilia A comprising a sterile preparation of a protein of claim 2 in admixture with a pharmaceutically accepted carrier.
14. A pharmaceutical preparation useful for therapeutic treatment of Hemophilia A comprising a sterile preparation of a protein of claim 3 in admixture with a pharmaceutically accepted carrier.
15. A pharmaceutical preparation useful for therapeutic treatment of Hemophilia A comprising a sterile preparation of a protein of claim 4 in admixture with a pharmaceutically accepted carrier.

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232

16. A method of treating Hemophilia A comprising administering to a patient an effective dose of the preparation of claim 12.
17. A method of treating Hemophilia A comprising administering to a patient an effective dose of the preparation of claim 13.
18. A method of treating Hemophilia A comprising administering to a patient an effective dose of the preparation of claim 14.
19. A method of treating Hemophilia A comprising administering to a patient an effective dose of the preparation of claim 15.

204

137

INTERNATIONAL SEARCH REPORT

International Application No PCT/US86/00774

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) ³

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC4: C12P 21/00, C12N 15/00, C12N 1/00, C07H 15/12.
A61K 37/00, A61K 37/02

II. FIELDS SEARCHED

Minimum Documentation Searched ⁴

Classification System	Classification Symbols
U.S.	435/68, 172.3, 240, 253, 255, 317 536/27; 530/383 514/12,8; 935/9,10,14,27,28,29,62

Documentation Searched other than Minimum Documentation
to the Extent that such Documents are Included in the Fields Searched ⁵

Computer Search CAS, Biosis, Medline 1975 to present:
Factor VIII, gene, cDNA, clone, recombinant, sequence

III. DOCUMENTS CONSIDERED TO BE RELEVANT ¹⁴

Category ⁶	Citation of Document, ¹⁵ with indication, where appropriate, of the relevant passages ¹⁷	Relevant to Claim No. ¹⁸
Y A	<u>Nature</u> , Volume 312, issued November 22, 1984 (LONDON, ENGLAND), (TOOLE ET AL..) "Molecular cloning of a cDNA encoding human antihaemophilic factor", pages 342-347, see page 345 in particular.	1,5,9-12, <u>16</u> 2-4,6-8, 13-15
Y A	<u>Nature</u> , Volume 312, issued November 22, 1984 (LONDON, ENGLAND), (GITSCHIER ET AL..) "Characterization of the human factor VIII gene", pages 326-330. see page 328 in particular.	1,5,9-12, <u>16;</u> 2-4,6-8,
Y A	<u>Nature</u> , Volume 312, issued November 12, 1984 (LONDON ENGLAND), (VEHAR ET AL..) "Structure of human factor VIII" pages 337-342, see page 339 in particular.	1,5,9-12, <u>16;</u> 2-4,6-8, 13-15

* Special categories of cited documents: ¹⁵

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search ⁹

05 July 1986

Date of Mailing of this International Search Report ⁹

10 JUL 1986

International Searching Authority ¹⁰

ISA/US

Signature of Authorized Officer ¹⁰

Robin L. Teskin
Robin L. Teskin

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, ¹⁶ with indication, where appropriate, of the relevant passages ¹⁷	Relevant to Claim No ¹⁸
A	<u>Blood</u> , Volume 83, Number 1, issued January 1984, (LONDON, ENGLAND), (KERNOFF ET AL.,) "Clinical experience with polyelectrolyte-fractionated porcine factor VIII concentrate in the treatment of hemophiliacs with antibodies to factor VIII, pages 31-41, see pages 38-40 in particular.	1-16
A	<u>Blood</u> , Volume 59, Number 3, issued March, 1982, (LONDON, ENGLAND), (FASS ET AL.,) "Monoclonal antibodies to porcine factor VIII coagulant and their use in the isolation of active cougulant protein", pages 594-600.	1-16
A	<u>Proceedings of the National Academy of Science, U.S.A.</u> , Volume 79, issued March 1982, (WASHINGTON, D.C. U.S.A.), (FULCHER ET AL.,) "Characterization of the human factor VIII procoagulant protein with a heterologous precipitating antibody", pages 1648-1652.	1-16
A	<u>Proceedings of the National Academy of Science, U.S.A.</u> , Vol. 79, issued December, 1982 (WASHINGTON, D.C., U.S.A.), (FAY ET AL.,) "Purification and characterization of a highly purified human factor VIII consisting of a single type of polypeptide chain". pages 7200-7204.	1-16
A	<u>Journal of Laboratory Clinical Medicine</u> , Volume 97, Number 1, issued January, 1981 (NEW YORK, CITY, NEW YORK, U.S.A.), (HOYER ET AL.), "The effect of thrombin on human factor VIII," pages 50-64.	1-16
A	<u>Blood</u> , Volume 59, Number 3, issued March 1982, (LONDON, ENGLAND), (KNUTSON ET AL.,) "Porcine Factor VIII: C prepared by affinity interaction with Von Willdebrnd Factor and Heterologous Antibodies: S-dium Dodecyl Sulfate Polyacrylamide Gel Analysis", pages 615-624.	1-16

REGULAR UTILITY

Form PTO-436
(Rev. 8/78)

PCT/US85/00774

SERIAL NUMBER <small>Serial No. 06/1979</small>	PATENT DATE			PATENT NUMBER		
725350	FILING DATE	CLASS	SUBCLASS	GROUP ART UNIT	EXAMINER	
06/725,350	04/12/85	435		127		

APPLICANT'S JOHN J. TOOLE JR., JAMAICA PLAIN, MA.

CONTINUING DATA
VERIFIED

REC'D 24 JUN 1986

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FOREIGN/PCT APPLICATIONS
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Foreign priority claimed 35 USC 119 conditions met	<input type="checkbox"/> yes <input checked="" type="checkbox"/> no	AS FILED	STATE OR COUNTRY	SHEETS DRAWGS.	TOTAL CLAIMS	INDEP. CLAIMS	FILING FEE RECEIVED	ATTORNEY'S DOCKET NO.
DAVID G. CONLIN	<input checked="" type="checkbox"/> yes	MA	8	8	2 3	300.00	15877	

Verified and Acknowledged: [Signature & Initials]

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Certifying Officer

Date: JUN 10 1986

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86/774

21 OCT 1986

Patent Cooperation Treaty (PCT)

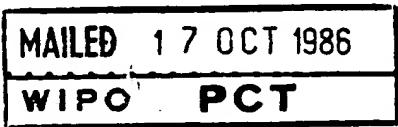
Copy of priority document furnished by the International Bureau pursuant to PCT, Rule 17.2(a).

Certificate

This is to certify that annexed hereto is a true copy from the certified copy of the priority application within the meaning of PCT Rule 17 received by the International Bureau in connection with the above-identified international application.

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Initial Copy

INTERNATIONAL APPLICATION
UNDER THE
PATENT COOPERATION TREATY
REQUEST

THE UNDERSIGNED REQUESTS THAT THE PRESENT
INTERNATIONAL APPLICATION BE PROCESSED
ACCORDING TO THE PATENT COOPERATION TREATY

(The following is to be filled in by the receiving Office) 10085

INTERNATIONAL
APPLICATION NO: 10085/T/US86/00774

INTERNATIONAL
FILING DATE:

11 APR 1986 010085

PCT INTERNATIONAL
APPLICATION

(Stamp)
Name of applying Office PCT International Application

Applicant's or Agent's File Reference
(indicated by applicant if desired) 5031-A-PCT

Box No. I TITLE OF INVENTION

Novel Procoagulant Proteins

Box No. II APPLICANT (WHETHER OR NOT ALSO INVENTOR); DESIGNATED STATES FOR WHICH HE/SHE/IT IS APPLICANT. Use this box for indicating the applicant or, if there are several applicants, one of them. If more than one person (includes, where applicable, a legal entity) is involved, continue in Box No. III.

The person identified in this box is (check one only): applicant and inventor* applicant only

Name and address:**

Genetics Institute, Inc.
87 Cambridge Park Drive
Cambridge, Massachusetts 02140
United States of America

Telephone number: (617) 876-1170 Telegraphic address:

Teleprinter address:

Country of nationality: United States of America Country of residence:*** United States of America

The person identified in this box is *applicant* for the purposes of (check one only):

all designated States all designated States except the United States of America the United States of America only the States indicated in the "Supplemental Box"

Box No. III FURTHER APPLICANTS, IF ANY; (FURTHER) INVENTORS, IF ANY; DESIGNATED STATES FOR WHICH THEY ARE APPLICANTS (IF APPLICABLE). A separate sub-box has to be filled in in respect of each person (includes, where applicable, a legal entity). If the following two sub-boxes are insufficient, continue in the "Supplemental Box," (giving there for each additional person the same indications as those requested in the following two sub-boxes) or by using a "continuation sheet."

The person identified in this sub-box is (check one only): applicant and inventor* applicant only inventor only*

Name and address:**

John J. Toole, Jr.
27 Lakeville Road
Jamaica Plain, Massachusetts 02130
United States of America

If the person identified in this sub-box is *applicant* (or *applicant and inventor*), indicate also:

Country of nationality: United States of America Country of residence:***

and whether that person is *applicant* for the purposes of (check one only):

all designated States all designated States except the United States of America the United States of America only the States indicated in the "Supplemental Box"

The person identified in this sub-box is (check one only): applicant and inventor* applicant only inventor only*

Name and address:**

197

If the person identified in this sub-box is *applicant* (or *applicant and inventor*), indicate also:

Country of nationality: Country of residence:***

and whether that person is *applicant* for the purposes of (check one only):

all designated States all designated States except the United States of America the United States of America only the States indicated in the "Supplemental Box"

* If the person indicated as "applicant and inventor" or as "inventor only" is not an *inventor* for the purposes of all the designated States, give the necessary indications in the "Supplemental box."

** Indicate the name of a natural person by giving his/her family name first followed by the given name(s). Indicate the name of a legal entity by its full official designation. In the address, include both the postal code (if any) and the country (name).

*** If residence is not indicated, it will be assumed that the country of residence is the same as the country indicated in the address.

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(i) if more than three persons are involved as applicants and/or inventors; in such case, write "Continuation of Box No. III" and indicate for each additional person the same type of information as required in Box No. III;

(ii) if, in Box No. II or any of the sub-boxes of Box No. III, the indication "the States indicated in the 'Supplemental Box,' is checked; in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the applicant(s) involved and, next to (each) such name, the country or countries (or EP or OA, if applicable) for the purposes of which he/she/it is applicant;

(iii) if, in Box No. II or any of the sub-boxes of Box No. III, a person indicated as "applicant and inventor" or "inventor only" is not inventor for the purposes of all designated States or for the purposes of the United States of America; in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the inventor and, next to such name, the country or countries (or EP or OA, if applicable) for the purposes of which the named person is inventor;

(iv) if there is more than one agent and their addresses are not the same; in such case, write "Continuation of Box No. IV" and indicate for each additional agent the same type of information as required in Box No. IV;

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(vi) if there are more than three earlier applications whose priority is claimed; in such case, indicate "Continuation of Box No. VI" and indicate for each additional earlier application the same type of information as required in Box No. VI;

(vii) if, in any of the Boxes, the space is insufficient to furnish all the information; in such case, write "Continuation of Box No. ..." (indicate the number of the Box) and furnish the information in the same manner as required according to the captions of the Box in which the space was insufficient.

CONTINUATION OF BOX NO. V
UNITED STATES OF AMERICA
12 APRIL 1985
725,350

198

123

If this Supplemental Box is not used, this sheet need not be included in the Request.

Box No. IV AGENT (IF ANY) OR COMMON REPRESENTATIVE (IF ANY); ADDRESS FOR NOTIFICATIONS (IN CERTAIN CASES) A common representative may be appointed only if there are several applicants and if no agent is or has been appointed; the common representative must be one of the applicants. The following person (includes, where applicable, a legal entity) is hereby/has been appointed as agent or common representative to act on behalf of the applicant(s) before the competent International Authorities:

Name and address, including postal code and country:

BERSTEIN, DAVID L.
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87 CAMBRIDGE PARK DRIVE
CAMBRIDGE, MASSACHUSETTS 02140
UNITED STATES OF AMERICA

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Telegraphic address:

Telex/teleprinter address:

Box No. V DESIGNATION OF STATES; POSSIBLE CHOICE OF EUROPEAN PATENT; POSSIBLE CHOICES OF CERTAIN KINDS OF PROTECTION OR TREATMENT. Where the name of a State is followed by two check boxes, either or both of the boxes may be checked. The checking of both boxes results in both a European and a national patent being requested for the same State. Designation of Switzerland includes designation of Liechtenstein (and vice-versa).

The following States are hereby designated:*** European Patent National Patent (if other national title or treatment desired, specify)**

AT Austria	<input type="checkbox"/>	<input type="checkbox"/> **
AU Australia	<input type="checkbox"/>	<input checked="" type="checkbox"/> **
BB Barbados	<input type="checkbox"/>	<input type="checkbox"/>
BE Belgium	<input type="checkbox"/>	[no national title available]
BG Bulgaria	<input type="checkbox"/>	<input type="checkbox"/> **
BR Brazil	<input type="checkbox"/>	<input type="checkbox"/> **
CH and LI Switzerland and Liechtenstein	<input type="checkbox"/>	<input type="checkbox"/>
DE Federal Republic of Germany	<input type="checkbox"/>	<input type="checkbox"/> **
DK Denmark	<input type="checkbox"/>	<input checked="" type="checkbox"/>
FI Finland	<input type="checkbox"/>	<input type="checkbox"/>
FR France	<input type="checkbox"/>	[no national title available]
GB United Kingdom	<input type="checkbox"/>	<input type="checkbox"/>
HU Hungary	<input type="checkbox"/>	<input type="checkbox"/>
IT Italy	<input type="checkbox"/>	[no national title available]
JP Japan	<input type="checkbox"/>	<input checked="" type="checkbox"/> **
KP Democratic People's Republic of Korea	<input type="checkbox"/>	<input type="checkbox"/>
KR Republic of Korea	<input type="checkbox"/>	<input type="checkbox"/> **
LK Sri Lanka	<input type="checkbox"/>	<input type="checkbox"/>
LU Luxembourg	<input type="checkbox"/>	<input type="checkbox"/> **
MC Monaco	<input type="checkbox"/>	<input type="checkbox"/> **
MG Madagascar	<input type="checkbox"/>	<input type="checkbox"/> **
MW Malawi	<input type="checkbox"/>	<input type="checkbox"/> **
NL Netherlands	<input type="checkbox"/>	<input type="checkbox"/>
NO Norway	<input type="checkbox"/>	<input type="checkbox"/>
RO Romania	<input type="checkbox"/>	<input type="checkbox"/>
SD Sudan	<input type="checkbox"/>	<input type="checkbox"/>
SE Sweden	<input type="checkbox"/>	<input type="checkbox"/>
SU Soviet Union	<input type="checkbox"/>	<input type="checkbox"/> **
US United States of America	<input type="checkbox"/>	<input checked="" type="checkbox"/> ** Continuation-in-part
EP all PCT Contracting States for which a European patent may be requested	<input checked="" type="checkbox"/>	**** these States are those listed above whose names are preceded by the codes AT, BE, CH and LI, DE, FR, GB, IT, LU, NL and SE
OA OAPI (Cameroon, Central African Republic, Chad, Congo, Gabon, Mali, Mauritania, Senegal, Togo)	<input type="checkbox"/>	OAPI Patent (if other OAPI title desired, specify)**

Space reserved for designating countries which become party to the PCT after the issuance of the present form (March 28, 1985): *124*

- * An address for the sending of notifications for a sole applicant or for a common representative may be indicated if no agent has been appointed to represent the applicant or, if there are several applicants, all of them.
- ** If another kind of protection or a title of addition is desired or if, in the United States of America, treatment as a continuation or a continuation in part is desired, indicate according to the instructions given in the Notes to Box No. V.
- *** The applicant's choice of the order of the designations may be indicated by checking the boxes of the designated States with sequential arabic numerals (see also the Notes to Box No. V).
- **** When this box is checked, none of the other boxes in the column "European patent" should be checked.

Box No. VI PRIORITY CLAIM (IF ANY). The priority of the following earlier application(s) is hereby claimed:

Country (country in which it was filed if national application; one of the countries for which it was filed if regional or international application)	Filing Date (day, month, year)	Application No.	Office of Filing (fill in only if the earlier application is an international application or a regional application)
(1) US	12 April 1985 <i>(12.04.85)</i>	725,350	
(2)			
(3)			

(Letter codes may be used to indicate country and/or Office of filing)

When the earlier application was filed with the Office which, for the purposes of the present international application, is the receiving Office, the applicant may, against payment of the required fee, ask the following:

the receiving Office is hereby requested to prepare and transmit to the International Bureau a certified copy of the above-mentioned earlier application/of the earlier applications identified above by the numbers (insert the applicable numbers) **725,350**

Box No. VII EARLIER SEARCH (IF ANY). Fill in where a search (international, international-type or other) by the International Searching Authority has already been requested (or completed) and the said Authority is now requested to base the international search, to the extent possible, on the results of the said earlier search. Identify such search or request either by reference to the relevant application (or the translation thereof) or by reference to the search request.

International application number or number and country (or regional Office) of other application:

US [Serial No.] 725,350

Date of request for search:

International/regional/national filing date

12 April 1985 *(12.04.85)*

Number (if available) given to search request:

Box No. VIII SIGNATURE OF APPLICANT(S) OR AGENTBruce M. Eisen

Bruce M. Eisen
Vice President
Chief Patent Counsel
Genetics Institute, Inc.

If the present Request form is signed on behalf of any applicant by an agent, a separate power of attorney appointing the agent and signed by the applicant is required. If in such case it is desired to make use of a general power of attorney (deposited with the receiving Office), a copy thereof must be attached to this form.

Box No. IX CHECK LIST (To be filled in by the Applicant)

This international application contains the following number of sheets:

1. request	3 sheets
2. description	23 sheets
3. claims	3 sheets
4. abstract	1 sheets
5. drawings	0 sheets
Total	30 sheets

Figure number of the drawings (if any) is suggested to accompany the abstract for publication.

This international application as filed is accompanied by the items checked below:

1. separate signed power of attorney
2. copy of general power of attorney
3. priority document(s) (see Box No. VI)
4. receipt of the fees paid or revenue stamps
5. cheque for the payment of fees
6. request to charge deposit account
7. other document (specify) Assignment; optional sheet re: deposited micro-organisms; cert'n - 1.12

(The following is to be filled in by the receiving Office)

1. Date of actual receipt of the purported international application: **19 Rec'd PCT/PTO 11 APR 1986**2. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application: **199**3. Date of timely receipt of the required corrections under Article 11 of the PCT: **125**4. Drawings Received No Drawings

(The following is to be filled in by the International Bureau)

Date of receipt of the record copy: **125**

APPLICANT Genetics Institute, Inc.		DOCKET NUMBER 5031-A-PCT	This column for use by receiving Office
RO/US RECEIPT DATE PCT/US86/00774	INTERNATIONAL APPLICATION NUMBER	SUBMISSION DATE	
UNITED STATES RECEIVING OFFICE FEE CALCULATION SHEET ¹			
FEES SUBMITTED OR AUTHORIZED:			
I. TRANSMITTAL FEE² 170 T <u>170.00</u> II. SEARCH FEE³ . . International Search to be conducted by (Check one) <input checked="" type="checkbox"/> ISA/US (US PTO) 250 S¹ <u>250.00</u> <input type="checkbox"/> ISA/EP (Eur. Pat. Off.) S² <u> </u>			
III. INTERNATIONAL FEE⁴ BASIC FEE⁵ Indicate the number of SHEETS contained in the international application <u>30</u> first <u>30</u> sheets 325 b₁ <u>325.00</u> remaining <u>0</u> sheets X \$ _____ = 0 b₂ <u> </u> (multiply excess over 30 by amount of supplement to Basic Fee) Add amounts entered in boxes b ₁ and b ₂ and enter total in box B. This figure is the amount of the BASIC FEE..... 325 B <u>325.00</u>			
DESIGNATION FEES⁶ Indicate the number of DESIGNATED STATES for which <u>National</u> patents have been sought and multiply by the amount of the designation fee <u>4</u> X \$ <u>80</u> = 320 d₁ <u>320.00</u> Indicate the number of GROUPS of designated STATES for which <u>regional</u> patents have been sought and multiply by the amount of the designation fee <u>1</u> X \$ <u>80</u> = 80 d₂ <u>80.00</u> Note instructions regarding the application of designation fees below— Add amounts entered in boxes d ₁ and d ₂ and enter total in box D. This figure is the amount of the DESIGNATION FEES 400 D <u>400.00</u>			
Add amounts entered in boxes B and D, and enter total in box I. This figure is the amount of the INTERNATIONAL FEE..... 725 I <u>725.00</u>			
IV. TOTAL FEES SUBMITTED OR AUTHORIZED: Add amounts entered in boxes T, S and I, and enter total in the total box. This figure is the total amount of the FEES SUBMITTED or AUTHORIZED..... 1145 TOTAL <u>1145.00</u>			
<i>Payment must be made in United States currency. Checks, postal money orders or bank drafts must be made payable to the Commissioner of Patents and Trademarks. Payment may also be made by authorization to charge to a Patent and Trademark Office deposit account.</i>			
DEPOSIT ACCOUNT AUTHORIZATION⁷ <input checked="" type="checkbox"/> The RO/US is hereby authorized to charged the total fees indicated above to my deposit account. <input checked="" type="checkbox"/> The RO/US is hereby authorized to charge any deficiency or credit any overpayment in the total fees indicated above to my deposit account. <input checked="" type="checkbox"/> The RO/US is hereby authorized to charge my deposit account for preparation, certification and transmittal of the priority document(s) identified in Box VI of the Request form.			
<u>07-1060</u> <i>Deposit Account Number</i>		<u>11 April 1986</u> <i>Date</i>	<u>David L. Bernstein</u> <i>Signature</i>
INSTRUCTIONS REGARDING DESIGNATION FEES: Use the space below to indicate, in order, those countries for which the designation fees submitted or authorized are to be applied. Include after the name of the country any indication that a regional patent is sought. If no countries are indicated below, the RO/US will apply the designation fees submitted or authorized to the designated countries in the order in which those countries are listed in the Request.			
		<u>201 124</u>	

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

Y
A

Nature, Volume 312, issued November 22, 1984, (LONDON, ENGLAND), (WOOD ET AL.,) "Expression of active human factor VIII from recombinant DNA clones" pages 330-336, see page 333 in particular.

1.5.9-
12,16;
2-4,6-8,
13-15

V. OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE ¹⁰

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. Claim numbers because they relate to subject matter ¹² not required to be searched by this Authority, namely:

2. Claim numbers, because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out ¹³, specifically:

VI. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING ¹¹

This International Searching Authority found multiple inventions in this international application as follows:

1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

- The additional search fees were accompanied by applicant's protest.
 No protest accompanied the payment of additional search fees.

194

234

010085

PATENT COOPERATION TREATY

INTERNATIONAL PUBLICATION No. WO86/06101
INTERNATIONAL APPLICATION No. PCT/US86/00774

NOTICE

INFORMING THE APPLICANT OF
THE COMMUNICATION OF THE
INTERNATIONAL APPLICATION
TO THE DESIGNATED OFFICES
issued under PCT RULE 47.1(c),
first sentence

To:

BERSTEIN, David, L.
Genetics Institute, Inc.
87 Cambridgepark Drive
Cambridge, MA 02140
ÉTATS-UNIS D'AMÉRIQUE

DATE OF MAILING OF THIS NOTICE
23 October 1986 (23.10.86)

APPLICANT'S OR AGENT'S
FILE REFERENCE
5031-A-PCT

From:
The International Bureau of WIPO
1211 Geneva 20
Switzerland

Notice is hereby given that the International Bureau has communicated, as provided in PCT Article 20, the international application referred to above to the following designated Offices on the date indicated above as the date of mailing of this Notice:

to the national Offices of AU, DK, JP, and to EP

Although the United States of America has been designated in the international application referred to above, that application was not communicated to the United States Patent and Trademark Office since that Office was the receiving Office of the said application and since the said Office has waived the requirement of communication provided for in PCT Article 20 for all international applications for which it is the receiving Office.

The applicant is reminded that he must enter the "national phase" before each designated Office by performing, within the time limit applicable under PCT Article 22 or 39(1), the acts referred to therein.

A copy of this Notice is being sent to each designated Office for its information under PCT Rule 47.1(c), third sentence.

Form PCT/IB/308 (June 1983)

214

T. Hirai
(Authorized Officer)

613

010085

11 April 1986
5031-A-PCT

Genetics Institute Inc.

International Application No: PCT/ /

MICROORGANISMSOptional Sheet in connection with the microorganism referred to on page _____, line _____ of the description ¹**A. IDENTIFICATION OF DEPOSIT¹**Further deposits are identified on an additional sheet ²Name of depositary institution ⁴

American Type Culture Collection

Address of depositary institution (including postal code and country) ⁴12301 Parklawn Drive
Rockville, Maryland 20852 USA

Name of Deposit	ATCC No.	Referred to on page/line	Date of Deposit
pSP64	39812	13/17-20	8/23/84
pDGR-2	53100	18/13	4/12/85

C. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE³ (if the indications are not for all designated States)**D. SEPARATE FURNISHING OF INDICATIONS⁴ (leave blank if not applicable)**The indications listed below will be submitted to the International Bureau later⁹ (Specify the general nature of the indications e.g., "Accession Number of Deposit")E. This sheet was received with the international application when filed (to be checked by the receiving Office)

(Authorized Officer)

 The date of receipt (from the applicant) by the International Bureau ¹⁰

was

213

(Authorized Officer)

International Application No: PCT/ /

MICROORGANISMSOptional Sheet in connection with the microorganism referred to on page _____, line _____ of the description ¹**A. IDENTIFICATION OF DEPOSIT²**Further deposits are identified on an additional sheet ³Name of depositary institution ⁴

American Type Culture Collection

Address of depositary institution (including postal code and country) ⁴12301 Parklawn Drive
Rockville, Maryland 20852 USA

Name of Deposit	ATCC No.	Referred to on page/line	Date of Deposit
pSP64	39812	13/17-20	8/23/84
pDGR-2	53100	18/13	4/12/85

C. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE⁵ (if the indications are not for all designated States)**D. SEPARATE FURNISHING OF INDICATIONS⁶ (leave blank if not applicable)**The indications listed below will be submitted to the International Bureau later⁶ (Specify the general nature of the indications e.g., "Accession Number of Deposit")E. This sheet was received with the international application when filed (to be checked by the receiving Office)

(Authorized Officer)

 The date of receipt (from the applicant) by the International Bureau is

was

(Authorized Officer)

DUPLICATE

010085

DAVID L. BERSTEIN
 GENETICS INSTITUTE, INC.
 87 CAMBRIDGE PARK DRIVE
 CAMBRIDGE, MASSACHUSETTS 02140

UNITED STATES DESIGNATED OFFICE
 (DO/US)

NOTIFICATION OF ACCEPTANCE
 UNDER 35 U.S.C. 371, 37 CFR 1.61

DATE OF MAILING *Released to
mail Room 11 Feb. 1987*

APPLICANT'S OR AGENT'S FILE REFERENCE

5031-A-PCT

IDENTIFICATION OF THE INTERNATIONAL APPLICATION

INTERNATIONAL APPLICATION NUMBER	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/US86/00774	11 APRIL 1986	12 APRIL 1985

APPLICANT FOR DO/US

TOOLE, JOHN J., JR.

NOTIFICATION

The above-identified application has met the requirements of 35 U.S.C. 371 and 37 CFR 1.61 and is ACCEPTED for national patentability examination in the United States Patent and Trademark Office.

The United States Serial Number assigned to the application and the relevant dates are:

U.S. Serial Number	11 APRIL 1986	11 APRIL 1986
	35 U.S.C. 102(e)	Date of receipt of
	Date	National Requirements

A request for immediate examination under 35 U.S.C. 371(f) was received on 09 DECEMBER 1986 and the application will be examined in turn.

No request for immediate examination under 35 U.S.C. 371(f) was received. The application will not be processed or examined before the time limit set forth in PCT Article 23.

202

127

UNITED STATES DESIGNATED OFFICE

ADDRESS ONLY:

COMMISSIONER OF PATENTS AND TRADEMARKS
 Box PCT
 Washington, D.C. 20231

AUTHORIZED OFFICER

Attn: DO/US

Mamie P. Person
 PCT INTERNATIONAL SERVICES DIVISION

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